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Manganese accumulation in the high pH microenvironment of *Phaeocystis* sp. (Haptophyceae) colonies from the North Sea

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ABSTRACT: Colonies of *Phaeocystis* sp. (Haptophyceae) isolated from the North Sea accumulated manganese during incubation of 15 d in a culture medium with near-natural ($0.45 \mu\text{mol l}^{-1} \text{ Mn}$) concentration. When maximum cell density was reached, up to 75 % of the manganese originally present in the medium was found in the colonies; the amount of manganese in the single cells remained at a constant level of about 7 %. Bio-accumulation of manganese in the mucous colonies appeared to be an indirect process: photosynthesis induced a pH above 9 inside the colonies – high enough for the transition of soluble Mn^{2+} to insoluble Mn-oxides. The role of *Phaeocystis* colonies in the cycling and transport of manganese in the North Sea can be expected to be important in view of their dominance in the phytoplankton, especially during spring.

INTRODUCTION

The regulation of the growth of *Phaeocystis* sp. – the algal species that dominates the plankton of the North Sea in spring, either in its colonial or its single-cell stage (Gieskes & Kraay 1975, 1977, Lancelot 1984) – has until now only been studied in relation to the presence of the macronutrients nitrate (Lancelot & Mathot 1985) and phosphate (van Bennekom et al. 1975, Veldhuis et al. 1986). No attention has been paid to trace elements such as manganese, iron, copper and molybdenum, although these are also essential nutrients. For example, manganese is an important element in the photosynthetic process of splitting water (O'Kelly 1968, Cheniae & Martin 1969). Evidence has recently been presented that low levels of manganese can limit the growth of several species of phytoplankton (Brand et al. 1983). Davidson & Marchant (1987) suggested that the abundance of a *Phaeocystis* strain from Prydz Bay, Antarctica, was regulated by manganese. According to these authors depletion of manganese from the surrounding seawater by colonies of this alga could even cause growth limitation of other phytoplankton species, which would explain why *Phaeocys-*

tis blooms are often associated with minimal co-occurrence of other species (Orton 1923, Smayda 1980).

There have been several investigations of the strong correlation between manganese oxidation and natural blooms of phytoplankton (Hunt & Smith 1980, Hunt 1983a). Sunda & Huntsman (1988) found that, in the ocean, phytoplankton influences the biological availability of manganese: in the southwestern Sargasso Sea the deep chlorophyll *a* maximum corresponded to the maximum of particulate manganese concentration and also to the maximum in the particulate manganese formation rate. Morris (1971) showed that during a *Phaeocystis* sp. bloom in the Menai Straits (N Wales, UK) the dissolved manganese concentration dropped, while the amount of particulate manganese dramatically increased; and Davidson & Marchant (1987) found that in experiments in vitro *Phaeocystis* is capable of an important manganese accumulation; in these experiments the concentration of manganese in *Phaeocystis* increased to up to 58 times that in the culture medium. They suggested that bacteria play an important role in the release of manganese back to the medium. On the other hand, others have found that bacteria can catalyze manganese oxidation (Kepkay & Neilson 1982, Rosson & Neilson 1982, Sunda & Huntsman 1987).

The brown colour of Antarctic *Phaeocystis* sp. col-

* Addressee for reprint requests

Table 1. PH-1 medium for culturing *Phaeocystis* sp. (S = 33.7 ‰). Major salts, Minor salts mix. I and Minor salts mix. II were heat-sterilized; vitamins were filter-sterilized (0.2 µm filter)

Major salts	Minor salts mix. I		Minor salts mix. II		Vitamins
NaCl	0.4190 M	Na ₂ EDTA	2.18 mg l ⁻¹	KBr	Biotin 0.5 µg l ⁻¹
MgCl ₂	0.0482 M	FeCl ₃ 6H ₂ O	1.575 mg l ⁻¹	SrCl ₂	Calcium-D-pantothenate (C ₁₈ H ₂₂ CaN ₂ O ₁₀) 50 µg l ⁻¹
CaCl ₂	0.0036 M	CuSO ₄ 7H ₂ O	5 µg l ⁻¹	AlCl ₃	Cyanocobalamin B12 10 µg l ⁻¹
Na ₂ SiO ₄	0.0227 M	ZnSO ₄ 7H ₂ O	11 µg l ⁻¹	RbCl	Folic acid 1 µg l ⁻¹
K ₂ SO ₄	0.0049 M	CoCl ₂ 6H ₂ O	5 µg l ⁻¹	LiCl	Inositol 2.5 mg l ⁻¹
NaHCO ₃	0.0024 M	MnCl ₂ 4H ₂ O	90 µg l ⁻¹	KI	Nicotinic acid (pyridine-3-carboxylic acid) 50 µg l ⁻¹
NaHPO ₄	4.0 µM	Na ₂ MoO ₄ 2H ₂ O	3 µg l ⁻¹	H ₃ BO ₃	Thiamine HCl 0.25 ml l ⁻¹
KNO ₃	100 µM				Thymine 1.5 mg l ⁻¹
Na ₂ SiO ₃	50 µM				

onies ascribed by Davidson & Marchant (1987) to the presence of manganese oxides has also been observed in *Phaeocystis* colonies in the North Sea (L. Venekamp pers. comm.). Therefore the hypothesis was formulated that *Phaeocystis* sp. from the North Sea is also capable of manganese depletion of the growing medium, namely by accumulation in the colonies. *Phaeocystis* colonies are in fact particle aggregates. It is known that a pH/Eh gradient builds up in aggregates of photosynthetically active cells (Richardson et al. 1988), in microbial mats (Revsbech et al. 1981, Jørgensen et al. 1983, Revsbech & Jørgensen 1983, 1986, Richardson & Castenholz 1987) and even around thalli of a freshwater alga (Okazaki & Tokita 1988). Because of a combination of high pH and high Eh in these micro-environments, soluble manganese must be expected to be transformed to insoluble manganese oxides (Garrels & Christ 1965).

METHODS

Cultures. The cultures used in these experiments were inoculated with *Phaeocystis* sp. cells (inoculation with ca 10⁵ cells l⁻¹ collected from the southern part of the North Sea in Dutch coastal waters). The taxonomic status of *Phaeocystis* is at present under debate (Sournia 1988). The cultures were not axenic. They were grown in PH-1 medium (Veldhuis & Admiraal 1987) with no nutrient replenishment (Table 1). In the North Sea, the concentration of dissolved manganese varies between 1.0 × 10⁻⁸ and 1.5 × 10⁻⁶ mol l⁻¹ (Duinker & Nolting 1978, Duinker et al. 1979, Kremling & Hydes 1988). The cultures were kept in 3 l glass bottles that were pre-cleaned with 6 N HCl (Pro Analyse, Merck) for 1 d to remove contaminants and rinsed several times with double distilled water. The bottles had silicon-rubber caps. Cultures were kept rotating (2 rpm) at 11°C. Light intensity was 100 µE m⁻² s⁻¹ (Philips no. 34 cool white fluorescent light; 14 h light, 10 h dark).

Sample manipulation (e.g. filtration) was performed in a laminar flow cabinet (class 100). On Days 4, 8, 11, 15 and 18 (6 h after the beginning of the light period) after inoculation, 5 ml and 500 ml samples were taken from the culture. For counting purposes the 5 ml samples were fixed with Lugol's Iodine; after sedimentation in a counting vessel, cell counts (number of single cells, number and volume of colonies, number of colonial cells) were carried out with an inverted microscope in 30 fields. Sample variance was less than 25 % of the main value.

The colonies and single cells in the 500 ml samples were separated from each other by 20 µm plankton gauze. The filtrate was filtered over polysulphonate filter (Gelman), 0.2 µm nominal pore size, to separate single cells from the culture medium. All filtrates were collected in acid-cleaned polycarbonate test tubes, then

Table 2. Measurements of manganese concentration in the 2 standards (CASS-1: $2.27 \pm 0.17 \mu\text{g Mn l}^{-1}$; SP1: $60 \pm 2 \mu\text{g Mn g}^{-1}$)

Day	Expt 1		Expt 2	
	CASS-1 ($\mu\text{g l}^{-1}$)	SP1 ($\mu\text{g g}^{-1}$)	CASS-1 ($\mu\text{g l}^{-1}$)	SP1 ($\mu\text{g g}^{-1}$)
4	2.01	52.2	2.30	61.5
8	2.20	60.0	2.53	69.3
11	2.45	62.3	2.16	64.3
15	2.39	56.4	2.05	65.1
18	nd	nd	2.23	55.2

acidified with Suprapur HNO_3 (Merck) to pH 2 to keep all manganese in solution. The tubes were kept in a refrigerator at 6° . Cultures were sampled each sampling day, as was also a non-inoculated 3 l bottle of culture medium (500 ml samples). These control samples were treated in the same way as the culture samples.

Analysis. The plankton gauzes and the filters with respectively the colonies and single cells of *Phaeocystis* sp. were dried, weighed and destructed, each with 5 ml Suprapur HNO_3 (1 h reflux boiling). To each series of samples (of one sampling day) 2 reference samples were added: the culture medium with CASS-1 (NRCC 1985), which contains $2.27 \mu\text{g Mn l}^{-1}$, and the algal material with SP1 (IAEA 1978) which contains $60 \mu\text{g Mn g}^{-1}$. Results of manganese concentration measurements of the culture samples were corrected with the results of the standards (Table 2).

Manganese concentrations were measured with a Perkin Elmer atomic absorption spectrophotometer (P.E. type 5000, equipped with a graphite furnace HGA 400, an autosampler AS40 and Data Station PE 10) with D_2 background correction. Sample size was $20 \mu\text{l}$; $10 \mu\text{l}$ matrix modifier ($0.01 \text{ mol l}^{-1} \text{HNO}_3$) was added to the culture medium samples to reduce interference with high salt concentrations. The temperature program used to measure the manganese concentration was 120/5/30, 220/5/5, 800/15/30, 2700/0/4, 2700/0/0 (temperature in $^\circ\text{C}$ / ramp time in s / hold time in s).

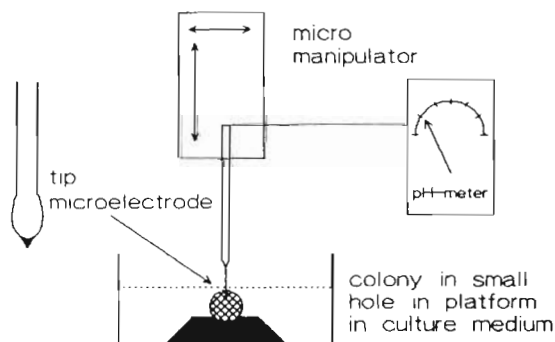


Fig. 1. Set up for measuring pH in *Phaeocystis* sp. colonies with a microelectrode

pH in colonies. The pH was measured inside and around colonies of *Phaeocystis* sp. with a specially shaped microelectrode (Fig. 1). This electrode had an active tip of about $200 \mu\text{m}$, with underneath a small, sharp nipple of about $25 \mu\text{m}$ in diameter to facilitate penetration and measure actually inside the colonies. A colony was placed on a small platform in a container filled with normal culture medium. After calibration with 4 different buffers (pH 7.0, 8.0, 9.0 and 10.0) the pH was measured during illumination of the colony with different light intensities (4 to $296 \mu\text{E m}^{-2} \text{s}^{-1}$). pH profiles were made to examine gradients of acidity inside a colony. All measurements were carried out at 12°C .

RESULTS

Manganese concentrations in *Phaeocystis* sp.

The growth rate, measured by following changes in cell numbers of the *Phaeocystis* culture, was maximum between Days 4 and 11 during Expt 1 (Fig. 2a); during Expt 2 between Days 1 and 4. On the last sampling day of Expt 2 there was a decrease in cell numbers (Fig. 2b). Measured in total volumes (single cells plus colonies), the culture was growing during the entire period in

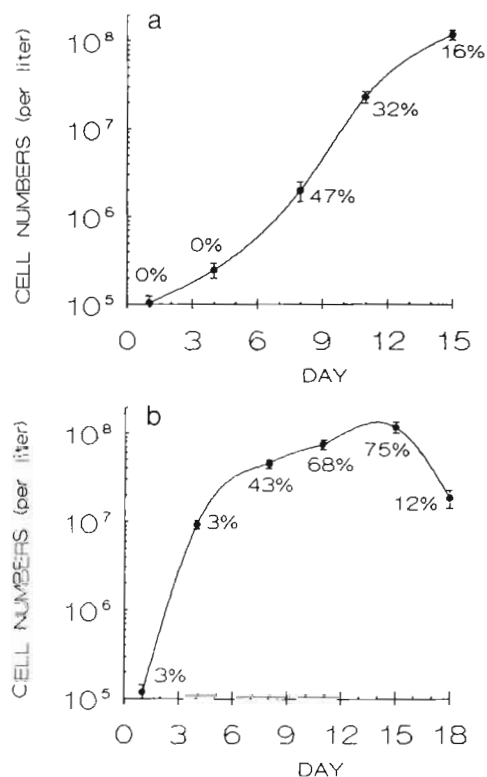


Fig. 2. *Phaeocystis* sp. Growth of culture in cell numbers. (a) Expt 1 (b) Expt 2. %: percentages of cells in colonies. Bars: standard error of cell counts

Table 3. Volumes per liter and manganese/volume ratios for single cell and colonies of *Phaeocystis*. nd: not determined

Day	Volume ($\text{mm}^3 \text{ l}^{-1}$) (\pm SD)		Ratio Mn/volume ($\mu\text{g}/\text{mm}^3$)	
	Single cells	Colonies	Single cells	Colonies
Expt 1				
1	0.007 (\pm 0.0013)	nd	nd	nd
4	0.016 (\pm 0.0032)	nd	232.5	nd
8	0.131 (\pm 0.0328)	1.113 (\pm 0.2783)	44.88	11.195
11	1.518 (\pm 0.2277)	12.769 (\pm 1.9154)	3.096	2.022
15	7.789 (\pm 0.9736)	54.996 (\pm 6.8745)	0.662	0.719
Expt 2				
1	0.007 (\pm 0.0015)	0.021 (\pm 0.0045)	nd	nd
4	0.589 (\pm 0.0636)	0.097 (\pm 0.0105)	3.769	30.722
8	1.702 (\pm 0.1872)	28.748 (\pm 3.1623)	1.645	0.816
11	1.558 (\pm 0.2057)	206.294 (\pm 27.231)	2.041	0.159
15	1.931 (\pm 0.2897)	324.182 (\pm 48.627)	2.123	0.083
18	1.086 (\pm 0.2606)	25.486 (\pm 6.1166)	3.517	0.66

Expt 1. For Days 1 and 4 no values are available for colony volumes because colonies were not yet present at these days. On the other sampling days the volume of the colonies was up to 8½ times the volume of single cells (Table 3). In Expt 2 the total volume of single cells remained at the same level after the rapid increase between Days 1 and 8. The total volume of colonies increased to approximately Day 15, after which it decreased again (Table 3). Apparently, the number of single cells did not increase any further after Day 8; in this period most of the growth activity was obviously associated with the colonial cells.

During the exponential growth phase of both experiments a strong decrease of manganese in the culture medium was found (Fig. 3). At maximum cell density only 15 % (Expt 1) and 27 % (Expt 2) of the original amount manganese remained in the medium. During both experiments the amount of manganese in the single cell fraction hardly changed, while at the same time there was a considerable increase in the number of single cells, especially in the Expt 1. In other words, the amount of manganese per single cell decreased considerably (Table 4). In the colony fraction the amount of manganese increased dramatically, up to 65 % (Expt 1) and 75 % (Expt 2) of the amount present originally in the culture medium. The chief difference between the experiments was the larger total volume of the colonies (Table 3) and the decrease in cell numbers (Fig. 2b) in Expt 2, which corresponds to the greater amount of dissolved manganese in the culture medium.

The amount of manganese per single cell in Expt 2 first decreased (up to Day 8), later remained at more or less the same level, then increased again (Table 4). The amount of manganese per colonial cell (= amount of manganese in the colonies divided by the number of colonial cells) was at a much higher level than in the single cells; it decreased until Day 15, and later increased again (Table 4). This increase was accompanied by an increase in dissolved manganese in the culture medium.

The manganese/volume ratio tended to decrease, in the colonies more than in the single cells (Table 3). In Expt 2, for single cells, this ratio decreased until Day 8, then remained approximately at the same level; after Day 15 a slight increase was registered. For the colonies, the manganese/volume ratio first decreased strongly (until Day 15), then increased again.

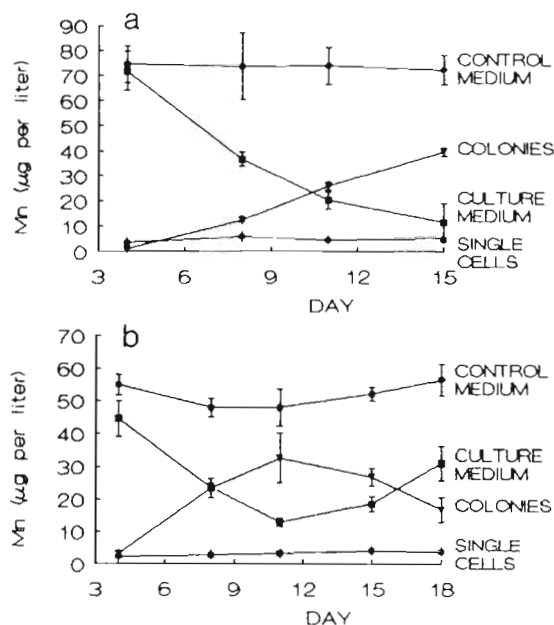


Fig. 3. *Phaeocystis* sp. Manganese concentration in culture medium, colonies, single cells and non-inoculated medium. (a) Expt 1; (b) Expt 2. Bars: standard error

Table 4. Manganese amount per single and colonial cell. nd: not determined

Day	Expt 1 Manganese (μg) per		Expt 2 Manganese (μg) per	
	single cell	colonial cell	single cell	colonial cell
4	7.5×10^{-6}	nd	2.461×10^{-7}	1.068×10^{-5}
8	1.47×10^{-6}	3.46×10^{-6}	1.08×10^{-7}	1.199×10^{-6}
11	1.013×10^{-7}	1.16×10^{-6}	1.316×10^{-7}	6.375×10^{-7}
15	2.14×10^{-8}	8.79×10^{-7}	1.372×10^{-7}	3.006×10^{-7}
18	nd	nd	2.309×10^{-7}	7.46×10^{-6}

pH in colonies

The first series of pH measurements – in a colony with a diameter of 2 mm, a flaky shape, and a light brown colour – were made at light intensities of 4, 50, 96, 195 and $296 \mu\text{E m}^{-2} \text{s}^{-1}$. The pH increased from 8.60 (the pH of the culture medium) to 8.90 at $96 \mu\text{E m}^{-2} \text{s}^{-1}$, remained at the same level up to $196 \mu\text{E m}^{-2} \text{s}^{-1}$ and decreased to 8.87 at $296 \mu\text{E m}^{-2} \text{s}^{-1}$. The second and third series of pH measurements were carried out with smaller light intensity intervals (steps of about $10 \mu\text{E m}^{-2} \text{s}^{-1}$) and with spherical colonies, which were 2 mm in diameter and coloured respectively brown and light brown and presumably younger than the colony of the first series. The pH in these young colonies increased from 8.56 to 9.11 and 8.95 at light intensities of respectively 71 and $79 \mu\text{E m}^{-2} \text{s}^{-1}$ (Fig. 4a). In the second colony a pH 'depth' profile was measured at a constant light intensity of $82 \mu\text{E m}^{-2} \text{s}^{-1}$. At all locations inside the colony the pH was the same (9.11), except perhaps between 0.1 and 0.3 mm below the outer membrane, where a slight increase to pH = 9.13 was recorded (Fig. 4b).

GEOCHEMISTRY OF MANGANESE AS IT AFFECTS MANGANESE UPTAKE OF ALGAL COLONIES

Dissolved manganese occurs in the valencies 2+, 6+ and 7+. Mn^{3+} and Mn^{4+} are also stable valencies in solids; however, in dissolved form these valencies have extremely low solubilities. Mn^{6+} and Mn^{7+} (manganate, MnO_4^- and permanganate, MnO_4^-) are only formed under extremely oxidizing conditions. These will not be considered here. In the 2+ form, which is the main dissolved form, the behaviour of manganese is comparable with Fe^{2+} and Zn^{2+} . The important complexes of Mn^{2+} are given in Table 5, together with their stability constants. The calculated speciation of manganese in the experimental medium (Table 1), at different pH values is given in Fig. 5. Complexes not given in this figure constituted less than 0.001 % of the dissolved manganese.

Manganese is capable of forming many different solid

oxides and hydroxides. The stable solid form in oxidizing environments in natural systems is $\delta\text{-MnO}_2$ (the mineral pyrolusite). However, thermodynamically unstable forms exist in nature. These forms have faster reaction kinetics than $\delta\text{-MnO}_2$ and can exist as a metastable solid for a long time. According to Hem & Lind (1983) the first precipitate when Mn^{2+} is oxidized is either Mn_3O_4 (hausmannite) or alpha-, beta- or gamma- MnOOH (groutite, feiknechtite or manganite, respectively). In the pH range of the experiment and in the North Sea, therefore, we expect that hausmannite will

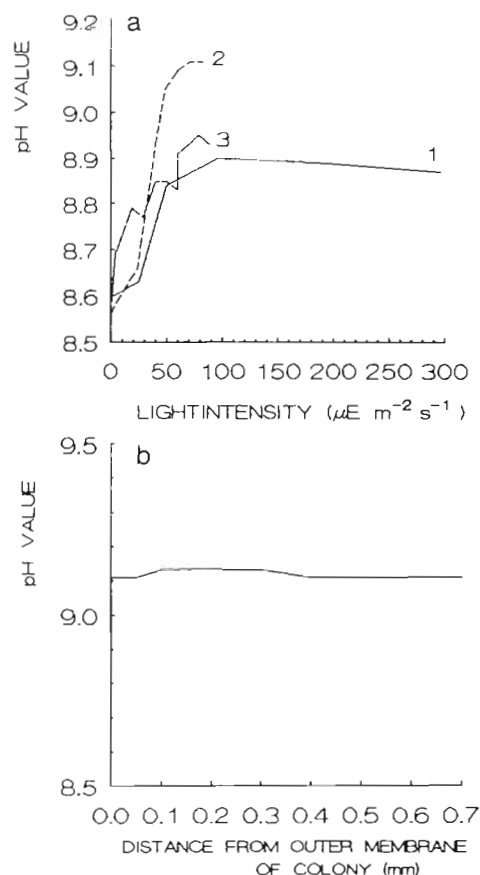


Fig. 4. *Phaeocystis* sp. pH values inside colonies. (a) pH inside 3 different colonies at changing light intensities (4 to $296 \mu\text{E m}^{-2} \text{s}^{-1}$). (b) pH 'depth' profile inside a colony at constant light intensity ($82 \mu\text{E m}^{-2} \text{s}^{-1}$)

Table 5. Complexation of manganese. K_f is the equilibrium constant for the formation of the complex

Complex (dissolved)	log (K_f)	Reaction	Source
$MnCl^+$	0.60	$Mn^{2+} + Cl^- \rightarrow MnCl^+$	Sadiq & Lindsay (1979)
$MnCl_2$	0.26	$Mn^{2+} + 2Cl^- \rightarrow MnCl_2$	Sadiq & Lindsay (1979)
$MnCO_3$	4.50	$Mn^{2+} + CO_3^{2-} \rightarrow MnCO_3$	Sadiq & Lindsay (1979)
$MnHCO_3^+$	1.25	$Mn^{2+} + HCO_3^- \rightarrow MnHCO_3^+$	Sadiq & Lindsay (1979)
$MnOH^+$	3.05	$Mn^{2+} + OH^- \rightarrow MnOH^+$	Sadiq & Lindsay (1979)
$Mn(OH)_2$	5.80	$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2$	Turner et al. (1981)
$Mn(OH)_3^-$	7.78	$Mn^{2+} + 3OH^- \rightarrow Mn(OH)_3^-$	Sadiq & Lindsay (1979)
$MnSO_4$	2.25	$Mn^{2+} + SO_4^{2-} \rightarrow MnSO_4$	Sadiq & Lindsay (1979)
$MnEDTA$	13.90	$Mn^{2+} + EDTA^{3-} \rightarrow MnEDTA$	Sposito & Mattigod (1980)
$MnHEDTA$	5.90	$Mn^{2+} + HEDTA^{2-} \rightarrow MnHEDTA$	Sposito & Mattigod (1980)

be the first precipitate. If manganese is in equilibrium with hausmannite, the dissolved concentration is calculated by us to be in the order of $0.4 \mu g Mn l^{-1}$ at a pH of 8.2 and an oxygen saturation of $10 mg O_2 l^{-1}$. This value is indeed close to the value found in the more offshore areas of the North Sea. Calculations for other forms of manganese oxide yield much lower dissolved concentrations. This suggests that hausmannite is probably the mineral controlling the solubility of manganese. In coastal areas higher concentrations, up to $25 \mu g l^{-1}$, are observed (Duinker & Nolting 1978, Duinker et al. 1979).

According to Hem (1981) the rate of precipitation (pptn) of hausmannite can be represented by the relation:

$$R_{pptn} = k_{pptn} \times A_{Mn_3O_4} \times S^n \quad (1)$$

where R_{pptn} = rate of crystal formation ($mol Mn d^{-1}$); k_{pptn} = rate constant ($mole Mn m^{-2} d^{-1}$); $A_{Mn_3O_4}$ = a measure of the availability of surface sites (m^2); and S =

the degree of supersaturation in solution (dimensionless). The exponent n represents the order of the reaction with respect to the solute, and was found to be 1.0 in the experiments performed by Hem (1981). The value of k_{pptn} which we calculated from the work of Hem (1981) has a high temperature dependency:

$$k_{pptn}(T) = k_{pptn}(20) \times k_T^{(T-20)} \quad (2)$$

where $k_{pptn}(20)$ is the rate constant at $20^\circ C$, its value being given by $p k_{pptn}(20) = 8.35$ (where $p = -\log_{10}$); k_T is the temperature dependency of rate constant = 1.20. S can be expressed as

$$S = (Q / K_{sp}) - 1 \quad (3)$$

where Q = the ion activity product for the formation of hausmannite; and K_{sp} = the thermodynamic solubility product. The reaction may stop before the activity quotient ratio (Q / K_{sp}) attains a value of 1, owing to an energy barrier that must be surmounted in the crystallization process. The ion-activity product Q can be written as

$$Q = (Mn^{2+}) \times (OH^-)^2 \times (O_2 aq)^{0.167} \quad (4)$$

where brackets denote activities rather than concentrations. For hausmannite, the value of pK_{sp} is 21.33 (Sadiq & Lindsay 1978). This equation, and the work of Hem (1981), shows that a rise in the pH of 1 unit will give a 100-fold increase in the rate of precipitation. On the other hand an increase in dissolved oxygen has only a marginal effect on Q . Calculations show that the original medium used in the experiments was already supersaturated towards hausmannite ($pQ = 20.27$, $pH = 8.0$). This however did not lead to any appreciable precipitation.

In the algal colonies the pH is 9.1, which will increase the precipitation rate by a factor of 160 (assuming manganese concentrations inside the algal colonies and outside are equal). In or around the single algal cells the pH can on theoretical grounds hardly be higher than in the medium; therefore no great increase in precipitation rate is found.

Differences in pH have a much larger effect on pre-

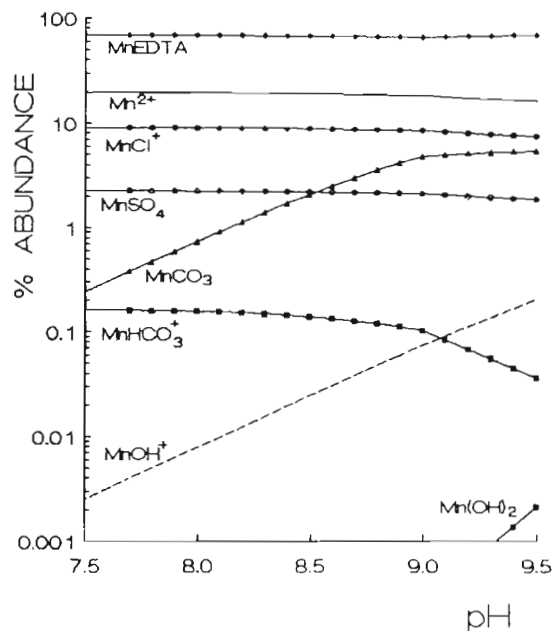


Fig. 5. Calculated speciation of manganese in the medium at different pH values

precipitation of manganese oxides than differences in oxygen concentration in the algal colonies, as can be seen from the ion-activity product Q . The precipitation of manganese oxides in the algal colonies can therefore be attributed primarily to the difference in pH which exists between the colonies and the medium.

The flux of manganese into the colonies can only be sustained by a gradient in manganese concentrations between the colonies and the medium. From Fick's first law the flux of manganese into the colonies can be calculated:

$$\text{Flux} = \frac{D_{\text{mol}} \times A_{\text{col}} \times (C_{\text{Mn-med}} - C_{\text{Mn-col}})}{\text{Diffusion distance}} \quad (5)$$

and D_{mol} (Mn^{2+}) (the diffusion coefficient) = $0.00008 \text{ m}^2 \text{ d}^{-1}$; A_{col} = surface area of the colonies; diffusion distance = distance between bulk medium and precipitates; $C_{\text{Mn-med}}$ = bulk medium manganese concentration; $C_{\text{Mn-col}}$ = manganese concentration in the colonies.

Assuming a distance of about 0.0005 m between the bulk medium and the precipitates this formula reduces to:

$$\text{Flux} \approx 0.15 \times A_{\text{col}} \times \Delta C \quad (6)$$

This flux is equal to the precipitation rate in the colonies. Since the flux to the colonies has been measured, the concentration of manganese inside the algae can be estimated. From this concentration the activity of the Mn^{2+} ion in the algae can be calculated, which is needed for Eq. (1). In this way it is possible to estimate the value of $(k_{\text{pptn}} \times A_{\text{Mn}_3\text{O}_4})$ of Eq. (1) inside the algal colonies. Finally, combining these results with the k_{pptn} from the work of Hem (1981) will give an estimate of the surface area of the precipitating manganese inside the algae, which will be an essential parameter when the effect of this precipitation process on the scavenging of other metals and phosphate from the seawater by adsorption is estimated. This however will need further detailed investigation.

DISCUSSION

Results show that colonies of *Phaeocystis* sp. can remove most of the manganese from the medium in which they grow. This removal is clearly related to the growth rate: the maximum increase in manganese in the colonies was recorded when the colony cells divided most rapidly (Figs. 2b and 3b).

During exponential growth of a culture, cell numbers increased at a higher rate than did cellular and colonial manganese. Throughout Expt 1 the amount of manganese per cell decreased, both in single and in colonial cells (Table 4). The difference between cells and colonies in the decrease of manganese concentration per

volume (Table 3) must be due to the presence of more manganese in a cell than in the same volume ($65.5 \mu\text{m}^3$) of colony. In other words, the concentration of manganese in the colonial matrix is low, but because there is so much more matrix than cells (more than 95 % of the volume of a colony is matrix), the largest amounts of manganese should be found in the colonies, not in the single cells outside the colonies. In Expt 2 the amount of manganese per single cell remained more or less constant, and even increased a little after Day 15; per colonial cell the amount of manganese first dropped, but after Day 15 increased again (Table 4). The increase in manganese per single and colonial cell after Day 15, when the culture was in its declining phase, was probably due to an increase of dissolved manganese in the culture medium: more manganese could be taken up by the remaining, living cells and colonies.

Phytoplankton remove dissolved manganese from their environment in 2 ways. Beside the direct process of taking up Mn^{2+} by the cells, there is an indirect process via the oxidation of Mn^{2+} to manganese oxides (Sunda & Huntsman 1985, 1986). The direct process is of importance in all species because manganese is a micronutrient that is essential to photosynthesis (O'Kelly 1968, Cheniae & Martin 1969). The amount of manganese in the fraction of single *Phaeocystis* sp. cells remained at approximately the same level, as expected. However, in the fraction of the colonies we registered a continuous increase of manganese (Fig. 3). This suggests that in *Phaeocystis* sp. the process of indirect removal, i.e. oxidation of manganese, dominates when the population is in its colonial phase. Oxidative transition to MnO_x takes place at a pH of about 9 and higher when the Eh is 0 to 0.7 V (Garrels & Christ 1965). In *Phaeocystis* colonies such high pH values are clearly produced by photosynthesis of the colonial cells (Fig. 4a). The pH (and also the Eh) around an algal cell increases following CO_2 consumption (and O_2 production). In the case of single cells a pH gradient is not built up easily due to turbulence. Cells in colonies, however, are bedded in a matrix of mucous polysaccharides. Because of the presence of this mucus the diffusion of photosynthetic products is slow and pH (or Eh) gradients consequently develop.

The increase of pH inside colonies is a photosynthesis-dependent process and therefore followed illumination, decreasing again at highest irradiance when photosynthesis was presumably light-inhibited (Fig. 4a). In the dark the pH decreased gradually, but there is no evidence that after a dark period the manganese concentration in the colonies had decreased again: Mn accumulation continued throughout the whole series of light-dark periods. Because of the 'nightly' decrease of pH there was probably some loss of colonial manganese during the dark periods, but this

amount must have been small compared to the amount of manganese that accumulated in the light periods. Dark manganese loss cannot be high because of the low rate of reduction of MnO_x to Mn^{2+} (Morgan 1967). Moreover, Mn^{2+} adsorbs to manganese oxides and to other particles (Kessick & Morgan 1975). In the fraction of the single cells the amount of manganese stayed at approximately the same level, in agreement with the notion that single cells are not able to create a strong pH gradient to transform Mn^{2+} to MnO_x .

Because of its dominance in the phytoplankton of the North Sea in spring, colonial *Phaeocystis* sp. no doubt plays an important role in the manganese cycle – and probably in cycles of other trace elements as well, such as zinc, iron and copper. However, *Phaeocystis* did not completely strip its culture environment of manganese, as has been suggested by Davidson & Marchant (1987). In fact, in their experiments 40 to 50 % of the manganese (to a level of $0.7 \mu\text{mol l}^{-1}$) remained in the culture medium. In our experiments this percentage was much lower: respectively 15 and 27 % (to a level of $0.21 \mu\text{mol l}^{-1}$; Fig. 3). In the North Sea even an accumulation of 85 % of manganese inside *Phaeocystis* colonies leaves enough for the growth of other algal species (Brand et al. 1983). It is likely that an equilibrium exists in the flux of manganese between cells, colony and culture medium and this equilibrium remains to be investigated.

The accumulation of manganese by *Phaeocystis* sp. no doubt influences transport of manganese in the southern North Sea. Due to the northward-directed residual current, the wax and wane of a *Phaeocystis* bloom in the North Sea is often separated in space and time, so the accumulated manganese can be transported in particulate form over large distances. The heaviest *Phaeocystis* blooms develop in the Dutch part of the southern North Sea (Gieskes & Kraay 1975, 1977). This material possibly accumulates in sedimentation areas such as the Frisian fronts zone, Oyster Grounds, and the Wadden Sea.

Other colony-forming algae probably also accumulate trace elements such as manganese because of manganese oxidation in their high pH microenvironment. Species with single cells or in simple chains are in a more dynamic relationship with manganese, as we found for single cells of *Phaeocystis* sp. The amount of particulate manganese in the North Sea can vary between 3.6×10^{-9} and $1.8 \times 10^{-7} \text{ mol l}^{-1}$ seawater (between 300 and 2000 $\mu\text{g Mn g}^{-1}$ suspended matter) (Duinker & Nolting 1976, 1977, Nolting 1986, Nolting & Eisma 1988). In the phytoplankton growing season a considerable part of this amount is probably fixed in colony-forming species such as *Phaeocystis* sp., *Chaetoceros socialis* and *Corymbellus aureus*.

We have not made any distinction between the sev-

eral different valency states of manganese. In further investigations it will be interesting to examine this in single cells and in the colonies. We have attempted a short trial experiment to show the presence of manganese oxides with higher states of valency by adding Leukoberbelin Blue (LBB; Krumbein & Altmann 1973, Rosson & Neilson 1982, Richardson et al. 1988) to cells and colonies, but no colour reaction was visible, possibly due to too strong dilution, or to too low acidity of the LBB due to the high colonial pH. Acidification by using 0.75 % instead of 0.25 % acidic acid in the reaction mixture may improve the situation (E. W. de Vrind-de Jong pers. comm.).

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